

THERAPEUTIC CALCIUM PHOSPHATE PARTICLES AND METHODS OF MANUFACTURE AND USE

This application claims benefit of the filing dates of U.S. Provisional Application Ser. Nos. 60/118,356; 60/118,364; and 60/118,355, all filed Feb. 3, 1999, the entire contents of each of which are hereby incorporated by reference.

BACKGROUND OF INVENTION

1. Field of the Invention

The present invention relates to novel calcium phosphate core particles, to methods of making them, and to methods of using them as vaccine adjuvants, as cores or carriers for biologically active material, and as controlled release matrices for biologically active material.

2. Description of Related Art

Nanometer scale particles have been proposed for use as carrier particles, as supports for biologically active molecules, such as proteins, and as decoy viruses. See U.S. Pat. Nos. 5,178,882; 5,219,577; 5,306,508; 5,334,394; 5,460,830; 5,460,831; 5,462,750; and 5,464,634, the entire contents of each of which are hereby incorporated by reference.

The particles disclosed in the above-referenced patents, however, are generally extremely small, in the 10–200 nm size range. Particles of this size are difficult to make with any degree of consistency, and their morphology is not described in any detail. None of these patents disclose the use of nanoparticles as sustained release matrices. Furthermore, these patents do not disclose the use of calcium phosphate particles as either (1) adjuvants for vaccines or viral decoys, or (2) controlled release matrices for delivery of pharmaceuticals or immunogenic materials.

There has been a suggestion in the literature to use calcium phosphate particles as vaccine adjuvants, but calcium phosphate particles have generally been considered an unsuitable alternative to other adjuvants due to inferior adjuvanting activity. See, e.g., Goto et al., *Vaccine*, vol. 15, no. 12/13 (1997). Moreover, the calcium phosphate evaluated was typically microparticulate (>1000 nm diameter) and possessed a rough and oblong morphology, in contrast to the core particles of the present invention.

Therefore, an important need remains for calcium phosphate core particles useful as core materials or carriers for biologically active moieties which can be produced simply and consistently. A further need remains for calcium phosphate core particles that can be effectively used as adjuvants for vaccines, as cores or carriers for biologically active molecules, and as controlled release matrices.

There is also a need for calcium phosphate core particles that can be effectively used as supports and matrices for sustained release of polynucleotide material (DNA or RNA) encoding immunogenic polypeptides. Traditional vaccination involves exposing a potential host to attenuated or killed pathogens, or immunogenic components thereof (e.g., proteins or glycoproteins). The basic strategy has changed little since the development of the first smallpox vaccine nearly a century ago, although modern developments permit genetic engineering of recombinant protein vaccines. However, traditional vaccine methodologies may be undesirable as a result of their expense, instability, poor immunogenicity, limited heterogeneity and potential infectivity.

Polynucleotide vaccination presents a different vaccine methodology, whereby polynucleotide material, such as

DNA or RNA, encoding an immunogenic polypeptide is delivered intracellularly to a potential host. The genetic material is taken up and expressed by these cells, leading to both a humoral and a cell-mediated immune response. It is not entirely clear whether DNA vaccines function as a result of integration or simply long-term episomal maintenance.

Polynucleotide vaccination provides numerous advantages over traditional vaccination. Polynucleotide vaccines eliminate the risk of infection associated with live attenuated viruses, yet advantageously induce both humoral and cell-mediated responses. Polynucleotide vaccines further provide prolonged immunogen expression, generating significant immunological memory and eliminating the need for multiple inoculations. Polynucleotide vaccines are very stable, permitting prolonged storage, transport and distribution under variable conditions. As a further advantage, a single polynucleotide vaccine may be engineered to provide multiple immunogenic polypeptides. Thus, a single DNA vaccine can be used to immunize against multiple pathogens, or multiple strains of the same pathogen. Finally, polynucleotide vaccines are much simpler and less expensive to manufacture than traditional vaccines.

Polynucleotide vaccines may take various forms. The genetic material can be provided, for example, in combination with adjuvants capable of stimulating the immune response. Administration of the DNA or RNA coated onto microscopic beads has been suggested. See J. J. Donnelly et al., *Annu. Rev. Immunol.* 15, 617 (1997). Various routes of administration are also possible, and may include, for example, intravenous, subcutaneous and intramuscular administration.

A desirable immune response to an immunogenic polypeptide is two-fold, involving both humoral and cellular-mediated immunity. The humoral component involves stimulation of B cells to product antibodies capable of recognizing extracellular pathogens, while the cell-mediated component involves T lymphocytes capable of recognizing intracellular pathogens. Cytotoxic T-lymphocytes (CTLs) play an important role in the latter, by lysing virally-infected or bacterially-infected cells. Specifically, CTLs possess receptors capable of recognizing foreign peptides associated with MHC class I and/or class II molecules. These peptides can be derived from endogenously synthesized foreign proteins, regardless of the protein's location or function within the pathogen. Thus, CTLs can recognize epitopes derived from conserved internal viral proteins (J. W. Yewdell et al., *Proc. Natl. Acad. Sci. (USA)* 82, 1785 (1985); A. R. M. Towsend, et al., *Cell* 44, 959 (1986); A. J., McMichael et al., *J. Gen. Virol.* 67, 719 (1986); A. R. M. Towsend and H., *Annu. Rev. Immunol.* 7, 601 (1989)) and may therefore permit heterologous protection against viruses with multiple serotypes or high mutation rates. Polynucleotide vaccination can stimulate both forms of immune response, and thus is very desirable.

Efforts to use polynucleotide vaccination have focused on the use of viral vectors to deliver polynucleotides to host cells. J. R. Bennink et al., 311, 578 (1984); J. R. Bennink and J. W. Yewdell, *Curr. Top. Microbiol. Immunol.* 163, 153 (1990); C. K. Stover et al., *Nature* 351, 456 (1991); A. Aldovini and R. A. Young, *Nature* 351, 479 (1991); R. Schafer et al., *J. Immunol.* 149, 53 (1992); C. S. Hahn et al., *Proc. Natl. Acad. Sci. (USA)* 89, 2679 (1992). However, this approach may be undesirable for several reasons. Retroviral vectors, for example, have restrictions on the size and structure of polypeptides that can be expressed as fusion proteins while maintaining the ability of the recombinant virus to replicate (A.D. Miller, *Curr. Top. Microbiol. Immu-*